

## CLAIMS

1. A method for producing a population enriched in a first type of cell from a mixture of at least three types of cells, said method comprising the steps of:

5 (a) providing a mixture of cells comprising at least first, second, and third types of cells;

(b) contacting said mixture with a first microfluidic channel that selectively allows the passage therethrough of said first type of cell to produce a first cell population enriched in said first type of cells relative to said second  
10 type of cell; and

(c) contacting said first cell population with a second microfluidic channel that selectively allows the passage therethrough of said first type of cell to produce a second cell population enriched in said first type of cell relative to said second and third types of cells, thereby producing said  
15 population enriched in said first type of cell.

2. The method of claim 1, wherein step (b) increases the relative population of said first type of cell relative to said second type of cell by a factor of at least 100, 1000, 10,000, 100,000, or 1,000,000.  
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3. The method of claim 1, wherein step (c) increases the relative population of said first type of cell relative to said third type of cell by a factor of at least 100, 1000, 10,000, 100,000, or 1,000,000.

25 4. The method of claim 1, further comprising step (d), arraying said second population of cells.

5. The method of claim 1, wherein said first type of cell is a fetal red blood cell, said second type of cell is a maternal red blood cell, and said third  
30 type of cell is a maternal white blood cell.

6. A microfluidic system for the separation of one or more desired cells from a sample, said system comprising:

(a) a lysis device comprising:

- 5 (i) at least two input channels;
- (ii) a reaction chamber; and
- (iii) an outlet channel,

wherein said input channels are connected to said outlet channel through said reaction chamber;

- 10 (b) a cell binding device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind a first type of cell, compared to a second type of cell; and

(c) an arraying device comprising a two dimensional array of locations for the containment of individual cells.

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7. A microfluidic system for the separation of one or more desired cells from a sample, said system comprising:

(a) a lysis device comprising:

- (i) at least two input channels;
- 20 (ii) a reaction chamber; and
- (iii) an outlet channel,

wherein said input channels are connected to said outlet channel through said reaction chamber; and

- 25 (b) a cell binding device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind a first type of cell, compared to a second type of cell.

8. A microfluidic system for the separation of a desired cell from a sample, said system comprising:

(a) a lysis device comprising:

(i) at least two input channels;

5 (ii) a reaction chamber; and

(iii) an outlet channel,

wherein said input channels are connected to said outlet channel through said reaction chamber; and

(b) an arraying device comprising a two dimensional array of locations  
10 for the containment of individual cells.

9. A microfluidic system for the separation of one or more desired cells from a sample, said system comprising:

(a) a cell binding device comprising obstacles disposed in a microfluidic  
15 channel, wherein said obstacles preferentially bind a first type of cell, compared to a second type of cell; and

(b) an arraying device comprising a two dimensional array of locations for the containment of individual cells.

20 10. The microfluidic system of any one of claims 6, 7, and 9, wherein said obstacles are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

25 11. The microfluidic system of any one of claims 6, 7, and 9, wherein said cell binding device further comprises obstacles that preferentially bind a third type of cell, compared to a fourth type of cell.

30 12. The microfluidic system of any one of claims 6, 8, and 9, wherein said arraying device further comprises actuators for the selective release of individual cells.

13. The microfluidic system of any one of claims 6-8, wherein said reaction chamber comprises a serpentine channel.

5           14. The microfluidic system of any one of claims 6-8, wherein said lysis device further comprises a dilution chamber and a third input channel, wherein said reaction chamber is connected to said outlet channel through said dilution chamber, and wherein said third input is disposed between said reaction chamber and said dilution chamber.

10           15. The microfluidic device of claim 14, wherein said dilution chamber comprises a serpentine channel.

15           16. A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

          (a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

20           (b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell;

          (c) collecting cells bound to said obstacles, thereby producing a cell population enriched in said second type of cell;

25           (d) arraying said cell population enriched in said second type of cell;

          (e) identifying one or more cells of said second type in said population enriched in said second type of cell; and

          (f) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

17. A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first, second, and third type and (ii) a solution that  
5 preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said third type of cell compared to said second type of cell;

10 (c) collecting cells not bound to said obstacles, thereby producing a cell population enriched in said second type of cell;

(d) arraying said cell population enriched in said second type of cell;

(e) identifying one or more cells of said second type in said cell population enriched in said second type of cell; and

15 (f) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

18. A method for producing a population of cells enriched in a second type of cell, said method comprising the steps of:

20 (a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device  
25 comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell; and

(c) collecting cells bound to said obstacles, thereby producing said population of cells enriched in said second type of cell.

19. A method for producing a population of cells enriched in a second type of cell, said method comprising the steps of:

5 (a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first, second, and third type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

10 (b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said third type of cell compared to said second type of cell; and

(c) collecting cells not bound to said obstacles, thereby producing said population of cells enriched in said second type of cell.

20. A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

15 (a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

20 (b) arraying the product of step (a);

(c) identifying said one or more cells of said second type; and

(d) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

21. A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

- 5 (a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said first type of cell compared to said second type of cell;
- (b) collecting cells not bound to said obstacles, thereby producing a depleted cell population;
- (c) arraying said depleted cell population;
- 10 (d) identifying said one or more cells of said second type in said depleted population; and
- (e) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

15 22. A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

- (a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell
- 20 compared to said first type of cell;
- (b) collecting cells bound to said obstacles, thereby producing a depleted cell population;
- (c) arraying said depleted cell population;
- (d) identifying said one or more cells of said second type in said
- 25 depleted population; and
- (e) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

23. The method of any one of claims 16-22, wherein said second type  
30 of cell is a fetal red blood cell.

24. The method of any one of claims 16-20, wherein said solution in step (a) comprises  $\text{NaHCO}_3$  and acetazolamide.

5           25. The method of any one of claims 16-20, further comprising the step, after step (a), of diluting the product of step (a) with a diluent in said one or more microfluidic channels.

10           26. The method of any one of claims 16-19, 21, and 22, wherein each of said obstacles is coated with a binding moiety.

15           27. The method of claim 16, wherein said binding moiety comprises an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

28. The method of any one of claims 16, 17, and 20-22, wherein at least 75%, 80%, 90%, 95%, 98%, or 99% of said one or more cells of said second type in said sample are arrayed in said arraying device.

20           29. The method of any one of claims 18-19, wherein at least 75%, 80%, 90%, 95%, 98%, or 99% of cells of said second type in said sample are collected.



30. A method of producing a cell population enriched in a second cell type, said method comprising the steps of:

(a) providing a sample comprising cells of at least a first and second type;

5 (b) introducing into one or more microfluidic channels (i) said sample; and (ii) a lysis buffer that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type, to yield said enriched population.

10 31. The method of claim 30, further comprising step (c), following step (b), of diluting said enriched population with a diluent in said one or more microfluidic channels to form a diluted sample..

15 32. The method of claim 31, further comprising step (d), following step (c), of collecting said diluted sample.

33. The method of claim 30, wherein said sample is introduced into two or more microfluidic channels.

20 34. The method of claim 30, wherein said first type of cell is a maternal red blood cell, and said second type of cell is a fetal red blood cell.

35. The method of claim 34, wherein said lysis buffer comprises  $\text{NaHCO}_3$  and acetazolamide.

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36. The method of claim 34, wherein said lysis buffer is in contact with said sample for at least 30 seconds.

30 37. The method of claim 30, wherein at least 98% of said second type of cells are not lysed.

38. A kit comprising:

(a) a microfluidic device comprising:

(i) at least three input channels;

5 (ii) a reaction chamber comprising a serpentine channel;

(iii) a dilution chamber comprising a serpentine channel; and

(iv) an outlet channel; and

(b) a lysis buffer that preferentially lyses one cell type over another.

10 39. The kit of claim 38, further comprising (c) a diluent.

40. The kit of claim 38, wherein said lysis buffer comprises  $\text{NaHCO}_3$  and acetazolamide.

15 41. The kit of claim 38, wherein said input channels and said outlet channel have diametric dimensions of between 10 and 500  $\mu\text{m}$ .

42. The kit of claim 41, wherein said serpentine channels of (ii) and (iii) have volumes of at least 10  $\mu\text{L}$  each.

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43. The kit of claim 42, wherein said serpentine channels of (ii) and (iii) have volumes of at least 20  $\mu\text{L}$  each.

25 44. A method of producing a depleted cell population, said method comprising the steps of:

(a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles that preferentially bind said first type of cell compared to said second type of cell; and

30 (b) collecting cells bound to said obstacles or collecting cells not bound to said obstacles, thereby producing a depleted cell population.

45. The method of claim 44, wherein said obstacles are coated with a binding moiety that binds to the surface of said first type of cell.

5           46. The method of claim 44, wherein said first type of cell is a fetal red blood cell.

47. The method of claim 44, wherein said second type of cell is a fetal red blood cell.

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48. The method of claim 44, wherein at least 60% of cells of said first type in said sample are bound to said obstacles.

49. The method of claim 44, wherein at least 70% of cells of said  
15 second type in said sample are not bound to said obstacles.

50. The method of claim 44, wherein said obstacles are ordered in a two-dimensional array.

20           51. A microfluidic device comprising obstacles disposed in a microfluidic channel, each of which is coated with a binding moiety.

52. The microfluidic device of claim 51, wherein said obstacles are ordered in a two-dimensional array.

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53. The microfluidic device of claim 51, wherein each of said obstacles is between 50 and 500  $\mu\text{m}$  high:

54. The microfluidic device of claim 51, wherein each of said obstacles  
30 has a dimension orthogonal to height that ranges between 5 and 500  $\mu\text{m}$ .

55. The microfluidic device of claim 51, wherein each of said obstacles is disposed at least 10 to 1000  $\mu\text{m}$  from any other obstacle.

5        56. The microfluidic device of claim 51, wherein said binding moiety comprises an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

57. A microfluidic device comprising:

10        (a) a first region of obstacles disposed in a microfluidic channel defining a fluid flow path, wherein said obstacles in said first region preferentially bind a first type of cell compared to a second type of cell; and

      (b) a second region of obstacles disposed in said microfluidic channel, wherein said obstacles in said second region preferentially bind a third type of  
15 cell compared to a fourth type of cell,  
      provided that said first and third types of cells are not the same.

58. The microfluidic device of claim 57, wherein said second and said  
fourth types of cells are the same.  
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59. The microfluidic device of claim 58, wherein said second and said third types of cells are the same.

60. The microfluidic device of claim 57, wherein said first region and  
25 said second region are arranged in series with regard to fluid flow in said microfluidic channel.

61. The microfluidic device of claim 57, wherein said first region and  
said second region are arranged in parallel with regard to fluid flow in said  
30 microfluidic channel.

62. The microfluidic device of claim 61, wherein said obstacles in said first region are interspersed among said obstacles in said second region.

5           63. A microfluidic device comprising:

          (a) a first region of obstacles disposed in a microfluidic channel defining a fluid flow path, wherein said obstacles in said first region preferentially bind a first type of cell compared to a second type of cell, wherein said obstacles are arranged in at least two substantially parallel rows and in at least two  
10       substantially parallel columns; and

          (b) a second region of obstacles disposed in said microfluidic channel, wherein said obstacles in said second region preferentially bind a third type of cell compared to a fourth type of cell, wherein said obstacles are arranged in at least two substantially parallel rows and in at least two substantially parallel  
15       columns,

          wherein the first and second regions are disposed adjacent one another in a microfluidic channel, and wherein the rows in the second region are displaced relative to the rows in the first region by a distance of less than the distance between the rows in the first region.

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          64. The microfluidic device of 63, wherein the obstacles in the first region are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

25           65. The microfluidic device of 63, wherein the obstacles in the second region are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

66. The microfluidic device of claim 63, wherein the ratio of the distance between the rows in the first region to the distance between the columns in the first region is about  $\sqrt{3}$ .

- 5        67. The microfluidic device of claim 63, wherein the ratio of the distance between the rows in the second region to the distance between the columns in the second region is about  $\sqrt{3}$ .